COLLOIDAL CHEMISTRY AND IMMUNOLOGY *

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In every immunologic research in vivo as in vitro, we shall always have to remember the fact that all the substances that we are treating (and considering) either are themselves colloidal substances, or are closely and inseparably connected with such.

Many investigators have emphasized also the great importance that colloidal chemistry has in biologic and immunologic chemistry, and there has been no lack of endeavors to explain every immunologic problem as colloidal. But some of these investigators—for instance, Traube[1]—have undoubtedly gone too far when they try to reduce immunology, including specificity, to pure surface processes, and such exaggerations have in many places deprived colloidal chemistry of credit.

At all events it has to be understood that the metabolism of the animal organism at the last instance is a purely chemical process, and tho the colloidal nature of the implicated substances cannot fail to produce an effect, the genuine chemical processes will take precedence. Speaking theoretically we must content ourselves with putting colloidal chemistry in the second place; but experience teaches us that where colloidal substances have been brought into contact with each other, it is their physical surface relations and electrical charges—that is, their colloidal state—which determines their quantitative relation, the chemical nature of the substances keeps in the background.

Consequently, biochemical investigations may reveal some quantitative facts belonging to the colloidal nature of the substances, but further than this we cannot as yet penetrate in spite of strong suspicions that some other process, possibly of a fermentative nature, cooperates tho hidden by the colloidal process. That is to say, studying immunologic problems we must always keep in mind the colloidal chemical relations, but we must not allow them too much weight, and whenever we have discovered what appears to be a colloidal chemical relation, we have always to ask ourselves whether this is not merely a shell that we have to crack in order to get at the real process.

* Received for publication April 23, 1916.

[1] Ztschr. f. Immuitätsf., 1911, 9, p. 246.

In the following pages I have endeavored to collect some facts and theories which may help to illustrate the somewhat obscure relations between colloidal chemistry and immunology.

I

The physical relations of the substances called colloids are best characterized as representing heterogeneous systems, that is, suspensions of solid particles in a fluid or emulsions of two fluids; but these mixtures are very intimate ones, and therefore the surfaces forming the limits between the mixed substances are extensive. But the relations of fluids at their surfaces and within their interiors are quite different. The surface of a fluid represents a tense elastic membrane, endeavoring to contract itself; this is the reason why a falling drop takes the globular form, the form in which the surface is reduced to a minimum. If I now dissolve a substance in the fluid, it will in most cases modify the surface tension, augmenting it or lessening it proportionately to the concentration. If now the dissolved substance lessens the surface tension, such being generally the case, this tension trying to get lessened draws a quantity of substance out of the surface of the fluid, thereby augmenting the surface concentration. The result may be rather a great difference between the concentration at the surface and that in the interior of the fluid.[2] On the surface the concentration may be so great that the substance will separate itself as a solid membrane, such as we may see at the surface of old urines or in old staining solutions.

But the surface tension does not exist only on the free surface of a fluid; it exists also on every surface forming the limit to another liquid or to a solid body. Here also we meet the augmenting concentration, and if the fluid moistens the limiting surface, the dissolved substance will fix itself on the solid body and so be eliminated from the liquid.

This is the phenomenon generally named adsorption, a process that is of great importance in all relations of colloidal chemistry. We see adsorption not only to solid bodies and to suspended and emulsified particles, but also to the smallest particles in the colloidal systems, and these particles, which themselves often can influence the surface tension, may be absorbed to solid bodies, suspensions, emulsions, or other colloid particles.

So we may have a great many different processes in the colloidal systems, and in the very complicated liquids that form blood and the

[2] Gibbs: Thermodynamical Studies. Freundlich: Kapillarchemie, 1909.

fluids of the body, or that result from our cultivation of bacteria in liquid media; we may expect to encounter whole series of different adsorptions, one parallel to another or antagonistic to another, forming a complicated system.

The characteristic trace by the adsorption is that the inner mechanics of the system do not change. The colloid particles keep themselves as before, their number neither augmenting nor lessening. But we may see other processes in colloidal chemistry in which such a change takes place. So we have the coagulation, or better, precipitation of a colloidal system, in which a precipitate forms itself under some certain outside influence; and we have the solution of a precipitate with the result that a coarse suspension dissolves itself under formation of a colloid solution. In both instances we see a change in the inner mechanics of the system, the number of the particles changing, several small particles agglutinating into one, or one greater particle disintegrating into several smaller. I propose to collect those two processes —in their effect great contrasts but in their nature closely related— under the name of alternations. Colloidal processes then may be considered under these groups: Adsorption, a binding in a colloidal system without change in its degree of dispersion; and Alternation, a binding that effects a thorough change in the same. (The degree of dispersion is the relation between surface and cubic measure of the particle: the more finely dispersed the substance the greater this relation.)

II

Quantitatively we may express the law of adsorption by a formula:

$$x = k\frac{y}{(1-y)^{1/n}}$$

in which x represents the quantity of adsorbing matter; y the adsorbed part of the substance adsorbed to x, the total quantity of this equalling 1; 1-y the nonadsorbed part; k a constant factor, depending first on the unities applied to x and second on the nature of the substances, the temperature, etc.; and 1/n an exponential constant, always less than 1, generally between 0.8 and 0.1.

Generally the adsorption formula is expressed in another way. For example,

$$\frac{y}{x} = k'\left[\frac{1-y}{v}\right]^{1/n}$$

in which v indicates the volume in which the reaction takes place. In this form the formula expresses that the concentration of the adsorbed substance

(y) on the adsorbing substance, (x) — namely the quotient y/x — is proportional to the concentration of the remaining substance 1 — y/v potensated with 1/ñ. But if we always employ the identical volume in a series of experiments, the volume may be contained within a constant

$$k\left[=\frac{k'}{v^{1/n}}\right]$$

and may be neglected in the schematic formula. We then get

$$\frac{y}{x}=k\,(1-y)^{1/n}$$

but I have preferred to develop this formula with respect to x, thus getting the afore-mentioned formula.

It may be mentioned that most books and papers on colloidal chemistry use other letters for the quantities, as is also the case in my paper on diphtheria toxin and antitoxin. We find in the place of x, m as the adsorbing substance; in the place of y, x as the adsorbed part; in the place of 1, a as the original quantity of the substance which is adsorbed; and in the place of 1/n, q as the exponential constant. The formula then looks like this:

$$\frac{x}{m}=k\,(a-x)^{1/n} \quad \text{or} \quad \frac{x}{m}=k\,(a-x)^{q}$$

Sometimes a — x is expressed as C for the remaining concentration, thus giving

$$\frac{x}{m}=k\,C^{1/n}$$

and a — x may be named C_2 and x/m expressed as C_1 and so we ultimately get: $C_1=kC_2^{1/n}$.

But for the purely mathematical treatment of this formula it is more convenient to use the signs generally used in mathematics; that is, x, y, etc., for the variable quantities, and k, q, n, etc., for the constant ones. Also I have used y to signify the fraction of adsorbed substance, employing the original quantity as unity. In this way I have arranged to reduce quantities which are absolutely very different, to the same order, a procedure that makes the calculation and the comparison of the different figures in the different experiments much easier.

Besides the law of adsorption, another law must be mentioned, not a law of colloidal chemistry, but in form closely allied with the adsorption law and undoubtedly of importance in immunology. This is the law of partition, written thus:

$$x=k\,\frac{y}{1-y}$$

This law rules the partition of a dissolved substance between two fluids that do not mix, such as oil and water. In the form which I have given to the

formula, it indicates for instance the fraction (y) of a quantity of phenol (1) that would be extracted from the water by a certain quantity of oil (x).

Generally this law is written thus: $C_1 = kC_2$, which is analogous to the last form that I stated for the adsorption formula. The importance of this formula is that in certain cases it may be absolutely identical with the adsorption formula; that is, when the molecular weight of the dissolved substance is different in the two media.

Thus, the molecular weight of benzoic acid in a solution in benzol is double its molecular weight in water; that is to say, in benzol 2 molecules join together.

If I now to a solution of benzoic acid in benzol add a certain quantity of water (x), I shall get the formula:

$$C_{\text{water}} = kC_{\text{benzol}}^{1/2}$$

But if I now use the first-mentioned letters, I get:

$$C_{\text{water}} = \frac{y}{x} \quad \text{and} \quad C_{\text{benzol}} = \frac{1-y}{v} \quad \text{and thus} \quad \frac{y}{x} = k\left[\frac{1-y}{v}\right]^{1/2}$$

or, if I develop this formula with respect to x:

$$x = kv^{1/2}\frac{y}{(1-y)^{1/2}}$$

a formula that cannot be distinguished from the adsorption formula. The only difference is that in partition the exponential constant has to be a very simple fraction, whilst in adsorption it may have almost any value less than 1.

III

I shall now proceed to some of the endeavors made to register immunologic processes as adsorptions.

As to agglutination, Eisenberg and Volk[3] could establish that fixation to specific bacteria followed a formula

$$x = k\frac{y}{(1-y)^{0.667}}$$

k having the value 24.7 for typhoid agglutinins and 19 for cholera vibriones. Arrhenius and Morgenroth[4] in a study of the fixation of hemolytic amboceptor to blood corpuscles found exactly the same relations, 1/n equalling 0.667 (⅔), and k equalling 18.3 for ox-blood corpuscles and 39.4 for sheep blood corpuscles.

Now it ought to be mentioned that Arrhenius[5] does not consider these fixations as adsorptions, but insists that they be considered as partitions. Of course it is very possible that a constant $1/n = ⅔$

[3] Ztschr. f. Hyg. u. Infektionskrankh., 1902, 40, p. 155.
[4] Arb. a. d. k. Gsndhtsamte, 1904, 20, p. 559.
[5] Ibid. Also Immunochemie (1907) and later works.

might be interpreted in this sense. The agglutinin ought then to change its molecular weight from 1 to 1.5; that is 3 molecules of agglutinin unite, when they enter the bacterium, into 2. And the circumstance that in two different immune processes we find the same value, will of course not fail to give valor to this conception. But in spite of this, it appears to me somewhat artificial to look for an explanation outside of adsorption, which undoubtedly is the most natural explanation. But Arrhenius urges against the possibility of an adsorption some experiments by Madsen with the fixation of coli-agglutinin in which the constant 1/n varied considerably and once was greater than 1, being 1.25. This fact is hardly compatible with an adsorption, where 1/n has to be less than 1; but these experiments cannot any more be reconciled to the theory of partition; then the molecular weight ought to be as constant as the exponential constant in the adsorption formula, and we cannot admit a change in the molecular weight sometimes to the smaller $(1/n>1)$, and at other times to the greater, as when 1/n is less than 1. We have to presume that the experiments of Madsen were executed under special circumstances which influenced their course. As considered by Arrhenius they do not permit a final conclusion.

Lastly I should mention my own endeavors to explain the fixation of toxin to antitoxin as an adsorption.[6]

Zangger[7] and Bilz, Much and Sieber[8] have suggested physical relations as predominant in the fixation and neutralization of toxin by antitoxin. Whilst Zangger limits himself to general considerations, Bilz and his colaborators enter more into details. They studied the fixation of the toxin to suspended anorganic substances. As they found that such fixation, which is undoubtedly an adsorption, took place, and believed that they had found that the toxin was made harmless by this process, they constructed on these experiments a purely physical theory of the neutralization of toxin.

In my experiments I could not confirm these observations. I found the toxin adsorbed to an anorganic suspended substance quite as harmful to guinea-pigs as before. The only difference observed was that its resorbability was hindered and its effect therefore more locally limited.

But the adsorption of toxin and of antitoxin to hydroxid of iron I was able to follow in all its phases, and I found that it followed

[6] Ztschr. f. Hygiene, B. 71.
[7] Centralbl. f. Bakteriol., I, O., 1903, 34, p. 428.
[8] Behring's Beiträge, 1903, No. 10, p. 30.

strictly the adsorption formula. The adsorption of toxin gave 1/n equal to 0.278 and k equal to 15.4, whilst the fixation of toxin to antitoxin gave to the same constants the values of 0.312 and 118.2. Later I calculated a number of other experiments by the adsorption formula and generally found good agreement especially in the last part of the curve where the toxin is saturated from 50 to 100%. In the first part of the curve the agreement is not always good, sometimes rather bad; but the method for determining the neutralization of the toxin in this part of the curve (up to 50%) is so inaccurate that we can hardly expect an exact conformity with any formula.

Arrhenius also denies the adsorption nature of this process, for which he urges a purely chemical nature; namely, the application of the law of Guldberg and Waage. Against this conception we may plead the firmness of the fixation and its lack of reversibility, its successive fastening, and the phenomenon of Danysz. All these features are essentially characteristic of adsorptions and added to the recognized colloidal nature of toxin and antitoxin ought to make it exceedingly probable that the process is an adsorption.

But the adsorption as such does not neutralize the toxin. Here we have to look for something behind the colloidal chemistry. Two possibilities suggest themselves to me: a purely chemical binding within the colloid particle or a fermentative decomposition of the toxin brought about by the antitoxin in some specific way. Experiments to this effect have not as yet been made, and this part of the question remains open.

IV

As to adsorption in immunology it is thus possible to enumerate several facts indicating the importance of the process. But if we now take up the other colloidal process, the alternation, we find ourselves on a quite new ground. First, as mentioned, alternation is a new conception. Second, we have no researches whatever that try to register such processes as hemolysis in colloidal chemistry.

The most important example of alternation in immunology is hemolysis. The S-formed course of this process is well known and very characteristic. Many have endeavored to explain its nature by this course but the result has been far from satisfactory.

A curve such as this is however not without analogies. Simon[9] has communicated some researches concerning the precipitation of albuminous substances with metal salts, in which we meet with exactly the same curve.

[9] Arch. di fisiologia, 1910, 8, p. 373.

The theoretic formula of a curve such as that mentioned is

$$y = \frac{x^n}{x^n + k}$$

(This curve with n equalling 2 is known in mathematics as the witch of Agnesi; applied here n always is greater than 2 up to 10 and more.) In this formula y ought to be the degree of hemolysis, x the quantity of hemolysin, and k and n constants.

If I develop this formula to x I get

$$x = k \left\{ \frac{y}{1 - y} \right\}^{1/n}$$

A formula that as to its formals is closely related to the adsorption formula

$$x = k \frac{y}{(1 - y)^{1/n}}$$

The only difference is that in the alternation formula the exponential constant is applied to the whole fraction y/1—y instead of to the denominator only as in the adsorption formula.

This formal analogy to a well known colloidal chemical formula gives, a priori, a certain probability, first to the formula itself, and second to the conception of the processes whereby it may be applied as purely colloidal. I have calculated a great many experiments on hemolysis with this formula and it has always appeared that if the experiment allows the tracing of any regular curve at all, the alternation curve coincides excellently with it. The more exact the experiment, the closer the fitting.

The calculation of an experiment by the alternation formula is accomplished, as regards its principles, by the same method as was indicated by Ostwald for calculations with the adsorption formula. The method consists in replacing the direct values by their logarithms.

The adsorption formula

$$x = k \frac{y}{(1 - y)^{1/n}}$$

written in the common way

$$\frac{y}{x} = \frac{1}{k} (1 - y)^{1/n}$$

and logarithmated gives

$$\log \frac{y}{x} = \log \frac{1}{k} + \frac{1}{n} \log (1 - y).$$

But a formula like this is identical with the genuine formula of first degree:

$$u = a + bv$$

If I graph this formula, putting v as abscisses and u as ordinates, I get at a straight line; the tangent to the inclination angle of this line is equal to b. It will be seen that in logarithmic formula log y/x corresponds to u, b to 1/n, and log (1—y) to v. By drawing up log y/x and log (1—y) as ordinates and abscisses respectively I have the opportunity of finding 1/n by determining the tangent to the inclination angle.

The constant k has to be calculated in the ordinary way of equations.

Applying these principles to the alternation formula, I get

$$x = k\left[\frac{y}{1-y}\right]^{1/n}$$

or logarithmated:

$$\log x = \log k = \frac{1}{n}\log\left[\frac{y}{1-y}\right]$$

Here also I shall have to draw up the curve with log x and log $y/1-y$ as ordinates and abscisses respectively, 1/n being as before the tangent to the inclination. Here the calculation of k is much easier inasmuch as when y equals 0.5, $y/1-y$ always equals 1, k thus being equal to x.

V

I now shall bring to notice some experiments on hemolysis calculated by the alternation formula.

First, I should mention some experiments of Madsen and Teruuchi communicated by Arrhenius.[10] These experiments, which were made with great accuracy, represent the average of many single experiments. The course of the curve is therefore very regular, In Table 1 are the calculations by the alternation formula, in Chart 1 the logarithmic curve representing a straight line, and in Chart 2 the ordinary curve. The calculated curve is traced and the values found by Madsen and Teruuchi are indicated by crosses.

It will be seen that in the logarithmic curves there is for the first part of the curves a rather weak conformity; but in the genuine curve on Chart 2 no trace of disagreement can be found. The reason is that for the first and last parts of the curve a very slight change in y gives a very great effect in y/1—y; thus the disagreement in the logarithmic curve is only an apparent one, which does not exist in reality.

But the last two points have given too small values in the calculations; it might be said that when hemolysis is very near to 1, its determination is rather difficult; but it may be possible that the formula, which only asymptomatically nears the value of y to 1, whilst the

[10] Meddelanden fran k. vetnskapsakademins Nobelinstitut, Vol. 2, No. 39.

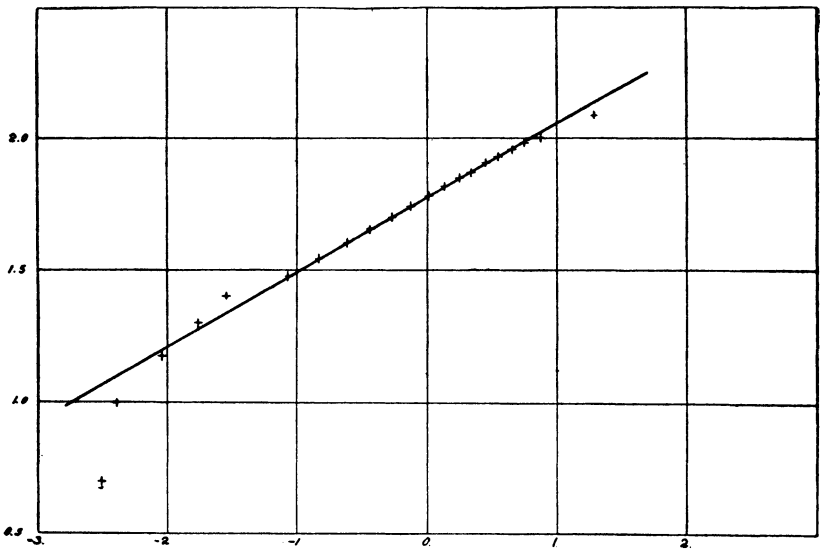

Chart 1

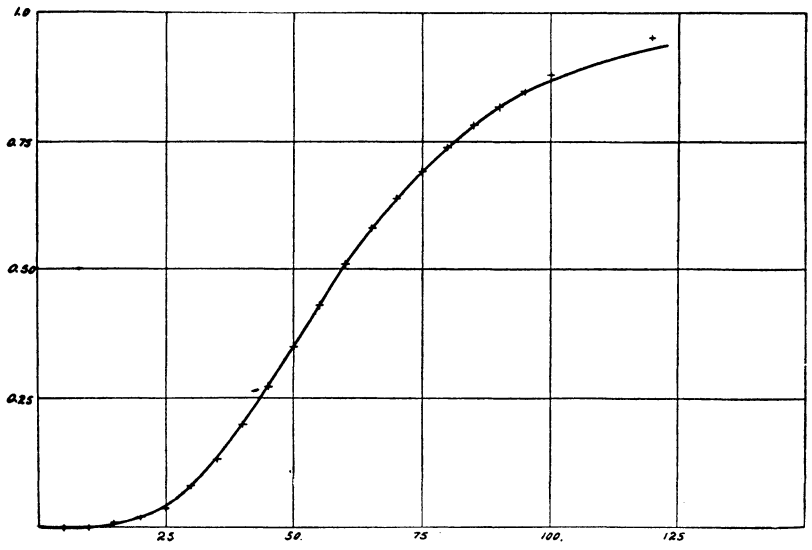

Chart 2

Charts 1 and 2. The logarithmic and ordinary curves for Madsen and Teruuchi's experiments on hemolysis calculated by the alternation formula. The values found by Madsen and Teruuchi are indicated by crosses.

hemolysis in reality gives a fairly well-determined value of x for y = 1, here needs correction.

Table 2 illustrates an experiment with amboceptor and complement executed by Leschly,[11] and in Charts 3 and 4 are represented the logarithmic and ordinary curves. Table 3 gives an experiment of

TABLE 1

HEMOLYSIS WITH VIBRIOLYSIN, ACCORDING TO MADSEN AND TERUUCHI

x	y Found	y Calculated	Log x	Log y/1-y Found	Log y/1-y Calculated
5	0.003	0.001	0.699	0.497—3	0.000—3
10	0.004	0.002	1.000	0.603—3	0.260—3
15	0.009	0.007	1.176	0.953—3	0.890—3
20	0.017	0.020	1.301	0.238—2	0.330—2
25	0.034	0.037	1.398	0.457—2	0.690—2
30	0.080	0.085	1.477	0.939—2	0.960—2
35	0.130	0.137	1.544	0.176—1	0.200—1
40	0.200	0.200	1.602	0.398—1	0.398—1
45	0.270	0.270	1.635	0.568—1	0.568—1
50	0.350	0.350	1.699	0.732—1	0.732—1
55	0.430	0.430	1.740	0.877—1	0.877—1
60	0.510	0.510	1.778	0.017	0.017
65	0.580	0.580	1.813	0.141	0.141
70	0.640	0.640	1.845	0.250	0.250
75	0.690	0.690	1.875	0.348	0.348
80	0.740	0.740	1.903	0.455	0.455
85	0.780	0.780	1.929	0.550	0.550
90	0.820	0.820	1.954	0.659	0.659
95	0.850	0.850	1.978	0.753	0.753
100	0.880	0.867	2.000	0.875	0.810
120	0.950	0.930	2.079	1.297	1.120

Vol. = 10 c.c. x = amount of lysin in cmm. y = degree of hemolysis. 1/n = 0.286

TABLE 2

HEMOLYSIS WITH COMPLEMENT ACCORDING TO LESCHLY, AVERAGE OF 4 DETERMINATIONS

x	y Found	y Calculated	Log x	Log y/1-y Found	Log y/1-y Calculated
0.006	0.110	0.054	0.788—3	0.093—1	0.75—2
0.007	0.160	0.107	0.845—3	0.281—1	0.08—1
0.008	0.200	0.190	0.903—3	0.398—1	0.37—1
0.009	0.280	0.282	0.953—3	0.590—1	0.61—1
0.010	0.400	0.407	0.000—2	0.824—1	0.83—1
0.011	0.500	0.503	0.042—2	0.000	0.06
0.012	0.610	0.625	0.079—2	0.194	0.22
0.013	0.680	0.716	0.114—2	0.328	0.40
0.014	0.800	0.807	0.147—2	0.602	0.57
0.016	0.890	0.890	0.204—2	0.909	0.91
0.018	0.930	0.930	0.255—2	1.128	1.13
0.020	0.950	0.954	0.301—2	1.279	1.32
0.022	0.970	0.972	0.343—2	1.510	1.54
0.024	0.980	0.980	0.380—2	1.695	1.70

x = amount of complement in c.cm. y = degree of hemolysis. 1/n = 0.191.

Teruuchi[12] with NaOH, the curves of which are traced in Charts 5 and 6.

There is a point in these curves that seems to confirm very strongly the correctness of the formula. It will be seen that in all of them the first amount of hemolysin does not have any apparent effect at all. Only after the addition of a certain quantity can we observe any hemo-

[11] Studier over Komplement, Aarhus, 1914, p. 22 (average of four determinations).

[12] Ztschr. f. Immunitätsf., 1909, 1, p. 351.

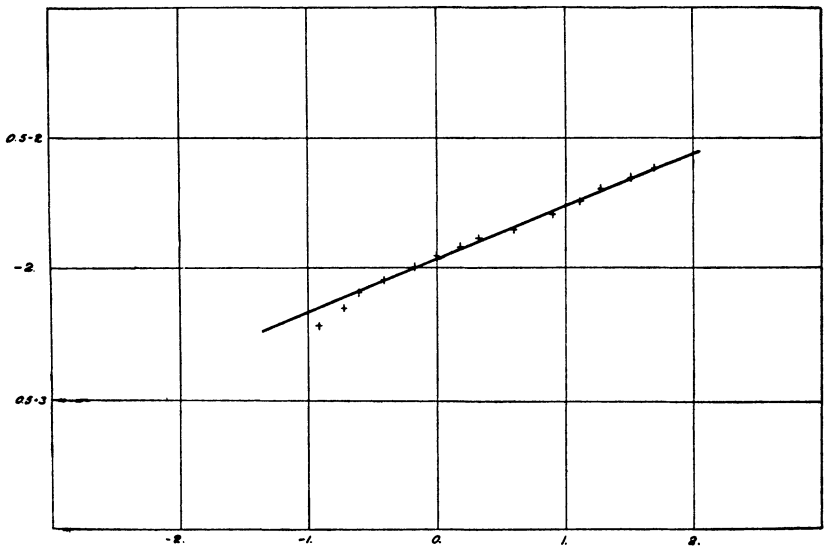

Chart 3

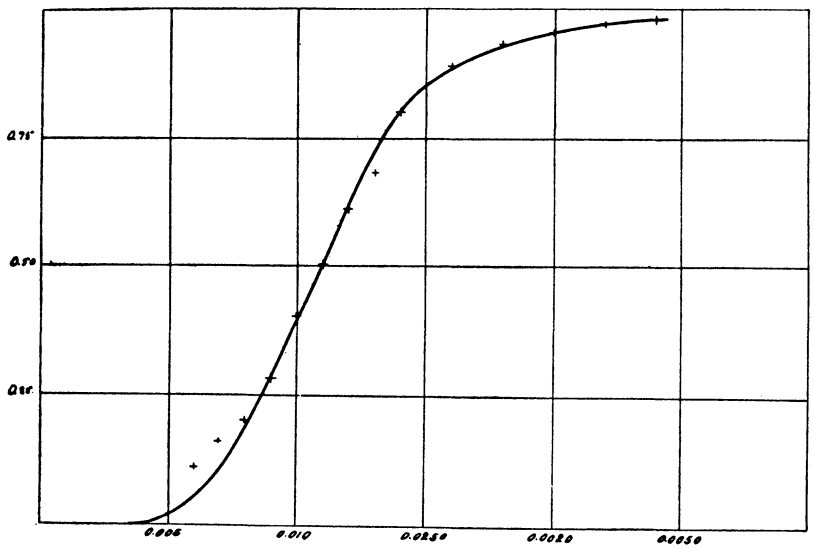

Chart 4

Charts 3 and 4. The logarithmic and ordinary curves for Leschly's experiments with amboceptor and complement.

lysis. This fact has been rather hard to explain, the investigators always believing that some foreign substance fixes some part of the hemolysin. With the adoption of the alternation formula this question disappears completely, the said course of the curve being explained by the particulars of the law.

But if we now proceed to the study of the velocity of the reaction of hemolysis, we encounter a fact that at first glance appears very strange. The velocity of reaction follows exactly the same law as the reaction with ascendent quantities of hemolysin. In Table 4 and in Charts 7 and 8 I have given the results of an experiment with n/100

TABLE 3

HEMOLYSIS WITH NaOH ACCORDING TO TERUUCHI

x	y Found	y Calculated	Log x	Log y/1-y Found	Log y/1-y Calculated
0.065	0.00	—	—	—	—
0.080	0.02	0.02	0.903—2	0.301—2	0.180—2
0.100	0.12	0.12	0.000—1	0.113—1	0.113—1
0.110	0.24	0.24	0.042—1	0.500—1	0.500—1
0.120	0.43	0.42	0.079—1	0.879—1	0.860—1
0.130	0.60	0.61	0.114—1	0.176	0.200
0.140	0.72	0.77	0.146—1	0.410	0.520
0.150	0.90	0.86	0.176—1	0.955	0.800
0.160	0.92	0.93	0.204—1	1.111	1.111
0.180	0.97	0.97	0.255—1	1.510	1.580
0.200	1.00	—	—	—	—

x = amount of NaOH. y = degree of hemolysis. 1/n = 0.1046.

TABLE 4

VELOCITY OF REACTION OF HEMOLYSIS WITH NaOH N/100

x	y Found	y Calculated	Log x	Log y/1-y Found	Log y/1-y Calculated
12	0.05	0.102	1.079	0.724—2	0.055—1
14	0.14	0.149	1.146	0.202—1	0.240—1
16	0.20	0.200	1.204	0.398—1	0.398—1
18	0.26	0.251	1.255	0.545—1	0.525—1
21	0.35	0.338	1.322	0.732—1	0.710—1
24	0.42	0.415	1.380	0.859—1	0.850—1
28	0.51	0.517	1.447	0.017	0.030
32	0.62	0.605	1.505	0.203	0.185
36	0.69	0.673	1.556	0.348	0.315
42	0.75	0.758	1.623	0.477	0.495
48	0.81	0.820	1.681	0.630	0.660
60	0.90	0.890	1.778	0.955	0.905

Temperature = 18°. x = time in minutes. y = degree of hemolysis. 1/n = 0.375.

NaOH, in which the conformity between found and calculated values is very remarkable. The only explanation of this is that equal amounts of hemolysin come into action in every unity of time.

This might perhaps be explained by some experiments made by Arrhenius, who found that the fixation of hemolysin to blood corpuscles follows the simple partition law, and he gives a table which shows a very good conformity to this law. This is very interesting because it is difficult to explain this fixation as an adsorption, which is a process that always takes place with great velocity, whilst the velocity

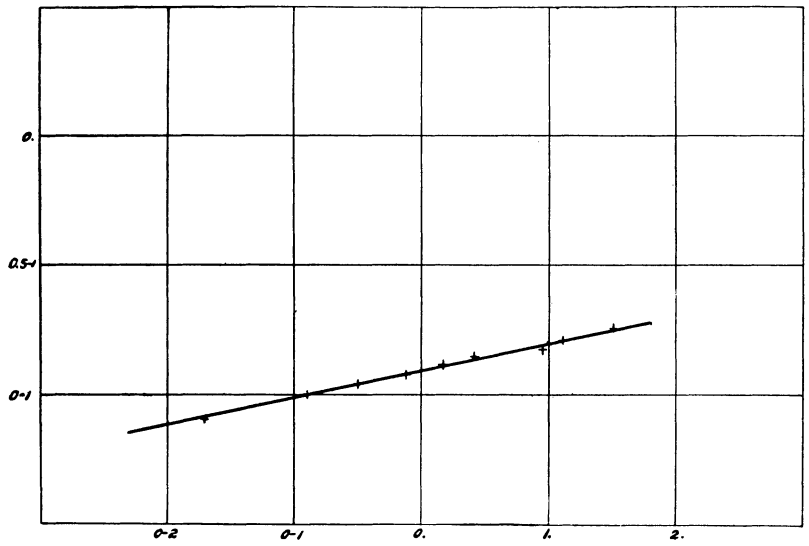

Chart 5

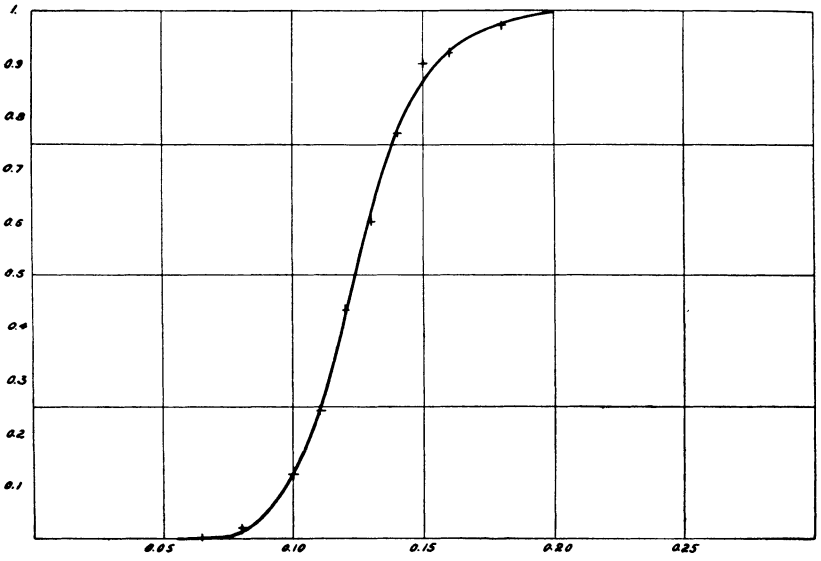

Chart 6

Charts 5 and 6. The logarithmic and ordinary curves for Teruuchi's experiments with NaOH.

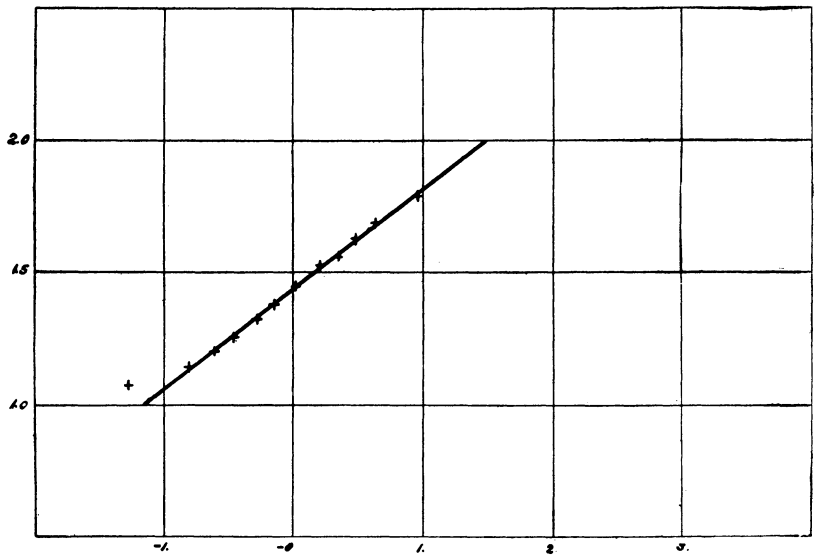

Chart 7

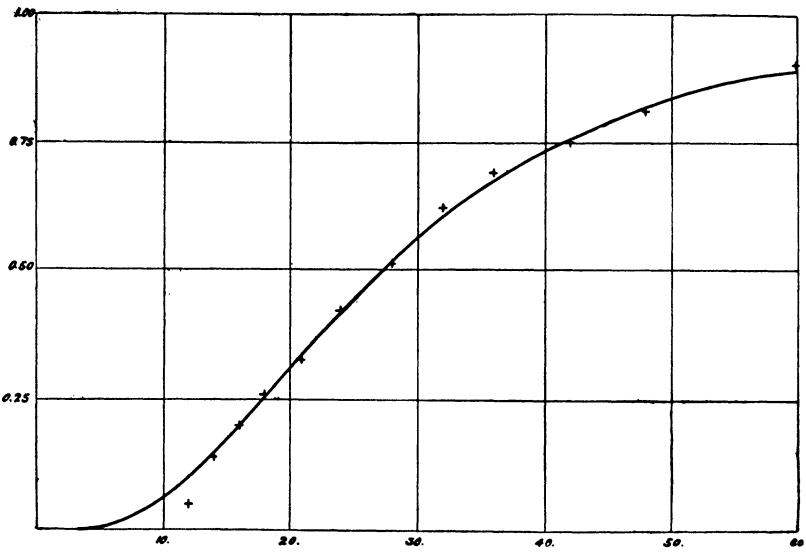

Chart 8

Charts 7 and 8. The logarithmic and ordinary curves for an experiment with n/100 NaOH.

of the binding of hemolysin, whether it is vibriolysin, complement, or NaOH, always takes place with a rather slow, at least very easily measured velocity.

As a result of these investigations of hemolysis it may be said that its quantitative parts are thoroughly explained by the alternation law. The nature of hemolysin is so far irrelevant and only gives some difference in the constants. That the alternation law is purely a colloidal chemical law, will now be shown by demonstrating its relation to the coagulation of albuminous substances.

VI

I shall communicate an experiment on coagulation (precipitation) of egg albumin with silver nitrate.

This reaction is well adapted to quantitative experiments, as the precipitation takes place very promptly, is at once complete, and, the precipitate formed being a very solid and heavy one, its further treatment on that account is easy. But in calculation we at once encounter the difficulty that the reaction is not a real one, but is made up of

TABLE 5

COAGULATION OF EGG ALBUMIN WITH $AgNO_3$

x'	y'	x	y Found	y Calculated	Log x	Log y/1-y Found	Log y/1-y Calculated
1.0	0.0204	—	—	—	—	—	—
2.0	0.0392	—	—	—	—	—	—
2.5	0.0498	—	—	—	—	—	—
3.0	0.0661	0.4	0.008	0.001	0.602—2	0.906—3	0.200—3
3.5	0.1032	0.9	0.100	0.050	0.954—1	0.045—1	0.750—2
4.0	0.1968	1.4	0.285	0.280	0.146	0.600—1	0.590—1
4.2	0.2777	1.6	0.440	0.440	0.204	0.893—1	0.893—1
4.4	0.3326	1.8	0.550	0.550	0.255	0.087	0.087
4.6	0.3874	2.0	0.660	0.660	0.301	0.288	0.288
4.8	0.4352	2.2	0.750	0.750	0.342	0.477	0.477
5.0	0.4712	2.4	0.820	0.816	0.380	0.695	0.640
5.6	0.5120	3.0	0.900	0.917	0.477	0.955	1.040
6.0	0.5360	3.4	0.950	0.954	0.531	1.279	1.320
6.5	0.5458	3.9	0.970	0.974	0.591	1.510	1.560
7.0	0.5558	4.4	0.990	0.986	0.643	1.996	1.830
8.0	0.5610	5.4	1.000	—	—	—	—

x' = c.c. $AgNO_3$ in 1.5% solution. x = x' — 2.6. y' = weight of precipitate.

$$y = \frac{y' - 0.0518}{0.561 - 0.0518} \qquad 1/n = 0.226.$$

TABLE 6

COAGULATION OF SERUM ALBUMIN WITH $CuSO_4$ IN SOLUTION 2/5 N

x	y Found	y Calculated	Log x	Log y/1-y Found	Log y/1y Calculated
0.0200	0.05	0.027	0.301—2	0.724—2	0.46—2
0.0233	0.07	0.078	0.367—2	0.824—2	0.93—2
0.0253	0.10	0.137	0.402—2	0.045—1	0.20—1
0.0266	0.18	0.162	0.425—2	0.342—1	0.32—1
0.0286	0.23	0.262	0.457—2	0.476—1	0.55—1
0.0300	0.42	0.334	0.477—2	0.859—1	0.70—1
0.0333	0.66	0.498	0.522—2	0.288	0.98—1
0.0366	0.70	0.643	0.564—2	0.368	0.26
0.0400	0.73	0.759	0.602—2	0.432	0.51
0.0533	0.95	0.958	0.729—2	1.279	1.36
0.0666	0.99	0.990	0.823—2	1.996	1.99

x = c.c. of solution. y = precipitate in fraction of the total precipitation. 1/n = 0.148.

purely chemical and colloidal chemical factors. We therefore find that the first part of the reaction is absolutely rectilinear, corresponding to a precipitation of silver chlorid and other chemical compounds of silver, and first after having saturated the substances that enter into chemical connections with the silver we find a real alternation. In the curve we must on this account subtract from the ordinate and from the abscissae the part that corresponds to the rectilinear part of the curve, and calculate only the rest of it. In Chart 9 I have shown the total curve that up to a value of x of 2.6, is perfectly straight. To this value of x corresponds an absolute $y = 0.0518$, and these values have

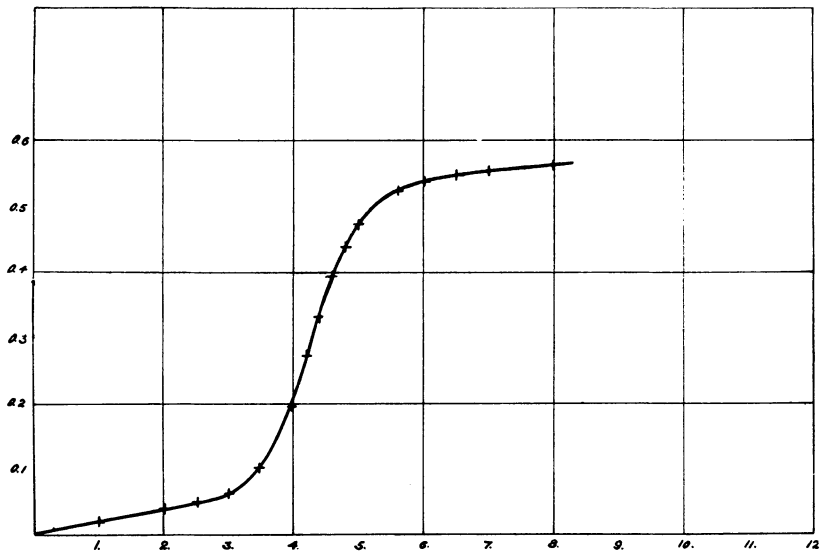

Chart 9. Curve for an experiment on coagulation (precipitation) of egg albumin with silver nitrate.

to be subtracted from x and y. The value of y got in this way has been divided with the value of the complete precipitation also reduced by subtraction so as to get the relative fraction of y.

The technic was as follows:

In a number of centrifuge tubes, the weight of which had been precisely determined, were placed increasing amounts of a solution of silver nitrate 1.5%. By experiment I found that 8 c.c. of this solution were sufficient for the complete precipitation of 10 c.c. of the solution of egg albumin. The volume of the silver solution was brought up to 10 c.c. in every tube and the 10 c.c. of the albumin solution added. After a few minutes the precipitate was well formed. The tubes were then centrifugated, the precipitate twice washed

with distilled water, which was decanted, and the tube dried for 24 hours at 120°. Ultimately the tube was weighed for the second time, and so the exact weight of the precipitate was determined.

It will be seen that the conformity between the calculated curve and the reduced experimental curve is a very close one, with the exception of the first point, where a value too great has been found for y. Probably here the last part of chemical precipitate mixes itself with the beginning of the colloid precipitation.

The precipitation of albumin solutions with copper salts has the advantage of being purely colloidal, but is not so regular as precipitation with silver salts. The reaction continues for a long time so that if we examine the precipitate at once we get entirely different values from those we should get after some hours. I refer to the experiment of Simon[9] with $CuSO_4$ and serum albumin. The conformity is not a very good one, but sufficient to prove the validity of the law (see Table 6 and Charts 12 and 13).

Agglutination is probably a purely colloidal phenomenon, and it would be very interesting if we should apply the alternation formula to it. But the technical difficulties of the determination are rather great, and as yet I have not attained any curves.

VII

There is a phase of agglutination as of hemolysis (particularly with NaOH) that ought to be considered. This is that the process is not concluded at a certain time, but runs along tho with decreasing velocity, as presumed by the formula of the velocity of reaction. The hemolysis with complement or with vibriolysin comes to a stop at half an hour at 37 C., but the hemolysis with NaOH shows a tendency to proceed for a long time. The result is that if I examine the hemolytic curve with increasing quantities of NaOH at different times, the curve shows a tendency to rise as shown in Chart 14. The curve begins with the form b and terminates by taking the form of a. In the case of agglutinations we find the same to be true.

Considered from a purely mathematical point of view, this signifies a change in the constants of the formula, both becoming smaller as the curve is rising.

This brings us up to the question of the sense of the constants. As related in the foregoing the constant k in the adsorption formula depends, first on the unity of x, second on the constant 1/n; but these being eliminated, it gives us a direct measure for the adsorption power

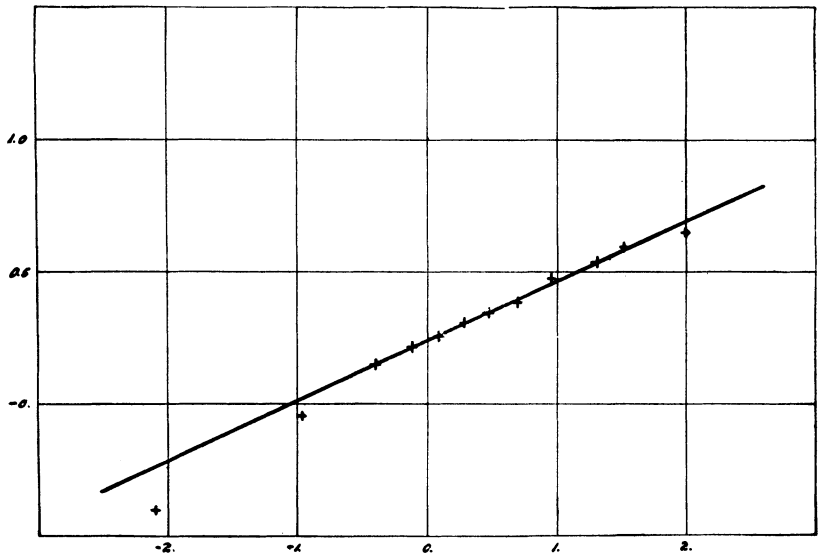

Chart 10

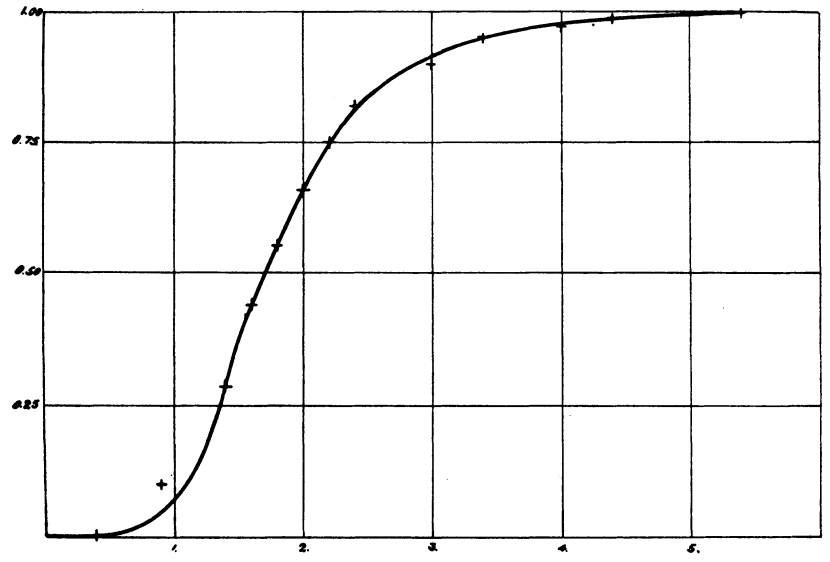

Chart 11

Charts 10 and 11. The calculated curve and the reduced experimental curve for an experiment on coagulation (precipitation) of egg albumin with silver nitrate.

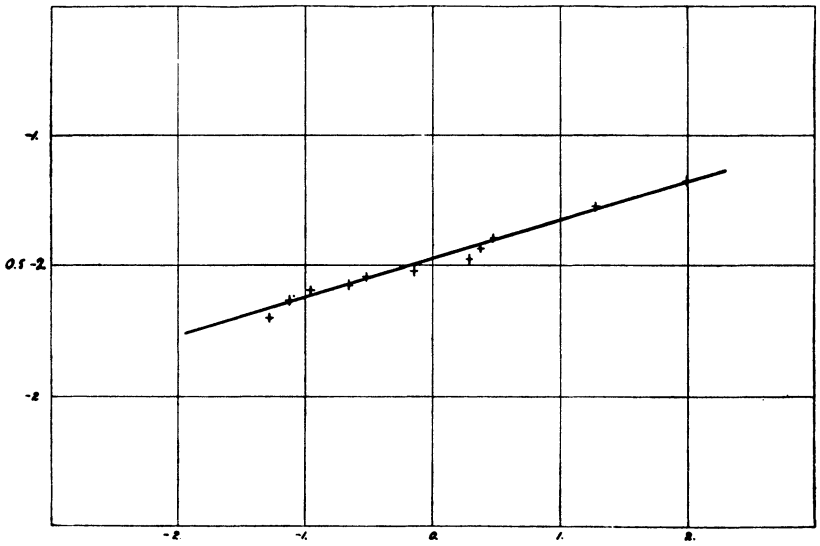

Chart 12

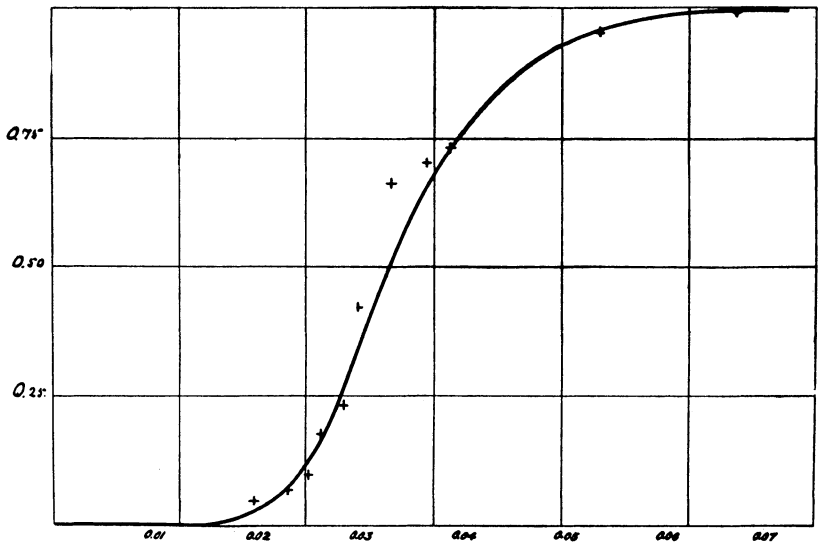

Chart 13

Charts 12 and 13. Curves for Simon's experiments with $CuSO_4$ and serum albumin.

of x. In the alternation formula it is entirely independent of 1/n and directly indicates the amount of x that gives $y = 0.50$.

But not so with 1/n. This constant that in the adsorption formula is of importance for the determination of the adsorptive power is in the alternation formula only an indicator of the difference between the amount of the hemolysin (or coagulating substance) that gives an alternation of 100% (approximately) and the amount that is apparently without effect. A greater 1/n (nearer to 1) gives a large difference and a more level course to the curve. A smaller 1/n (nearer to 0)

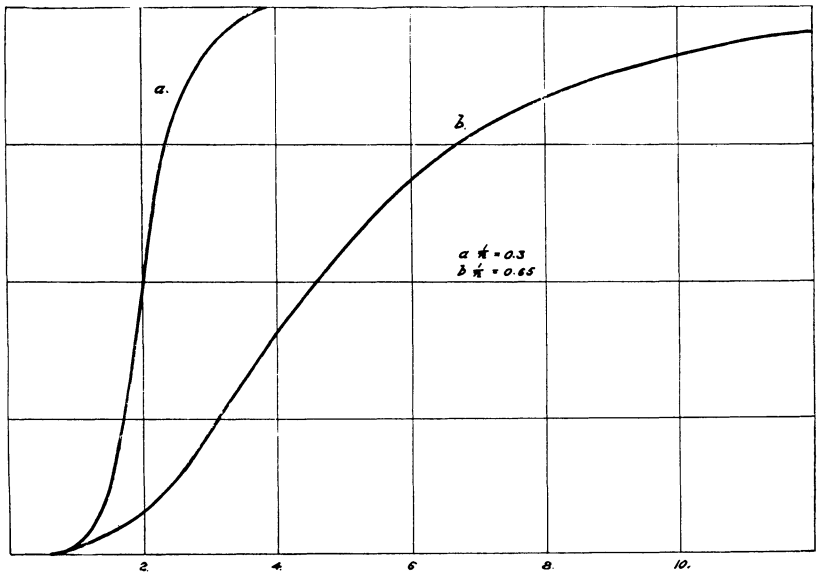

Chart 14. Curve of the progress of hemolysis with NaOH. The curve begins with the form b and terminates by taking the form a.

gives a steeper curve and a small difference between x for y=1 and the maximal value of x for $y = 0$. If with a constant k I trace a lot of theoretical curves with different 1/n, all these curves intercross in a point where $y = 0.5$ and $x = k$.

But as yet it has not been possible to find a plausible explanation of the constant 1/n. It might be an obvious conclusion that it has a relation to the degree of dispersion of the colloidal systems, but by trying to develop this theory I found myself entangled in such a net of mutually contradictory facts that it had to be abandoned. Neither has

the endeavor to relate the colloidal chemical laws to the law of Guldberg-Waage given any encouraging results.

But I suppose we shall not err when we interpret the whole of the immunologic processes in the matter of their quantitative relations as colloidal processes, following partially the adsorption law, partially the alternation law, with the exception of some few that follow the partition law. Theoretically we may think of other of the possibilities which may present themselves as analogous formulas, x for example:

$$x = \frac{y^{1/n}}{1-y} \qquad \text{and} \qquad x = \frac{y^{1/n}}{(1-y)^{1/q}}$$

but these possibilities have as yet not been examined. The first gives a curve with a turning point much nearer to the abscissae than the alternation curve, to which it has some resemblance; but I have as yet seen no process that is likely to follow it. The other is a curve of great flexibility, and it may be that it is in reality the curve of adsorption as well as of alternation, giving by slight differences in one of the exponential constants from the adsorption or alternation formula the corrective element which these formulas sometimes need to make them fit closely to the experiments. But the calculation of an experiment to this formula needs special resources, and so I have not been able to verify these possibilities.

VIII

But now we encounter a last question: Have we in reality solved the immunologic problem by referring fixation to colloidal formulas? And do these formulas satisfy the qualitative side of the question? I regret to say that I do not think so. First of all we get no explanation at all of specificity through the colloidal chemical theory. The adsorptions as well as the alternations are not specific processes. The adsorption of diphtheria toxin may happen to every positive colloid or suspension: but neutralization takes place by fixation to antitoxin only. We may have a nonspecific hemolysis for instance in NaOH and with vibriolysin, but that does not explain anything about the specific immune body, the amboceptor, and the necessity of its previous fixation to the blood corpuscle for hemolysis by complement, as little as the adsorption of the amboceptor to the blood corpuscle explains anything.

No endeavor at an explanation of specificity on the basis of colloidal chemistry has been successful, and the only thing that we know about specificity, namely, that it seems to be in some way connected with

aromatic aminoacids in the antigen molecule, is of a purely chemical, not colloïdal chemical, nature.

I think that a portion of the truth may be found in a series of facts brought to light in the last few years. This is the investigation of blood ferments.

Inaugurated in 1912 by Abderhalden[13] this investigation has been, as far as I have been able to find in literature, mostly the work of North-American investigators.[14]

Abderhalden ascertained that after injection of some substances there appeared in the blood ferments which could decompose the injected substance. This was the result not only with genuine antigens (albumins) but also with fats and carbohydrates.

The case has been carefully studied especially as to the effect of the albuminous substances. It appears that not only albumin foreign to the species may have the effect of provoking the fermentative effects in the serum, but that the entrance of albumin of the proper organism but foreign to the blood may have the same effect. For instance, we see ferment in the blood for the albumin of the placenta (known as the Abderhalden reaction) due to the transit of placental substances into the blood during pregnancy. Abderhalden himself was of the opinion that these ferments were formed under the influence of the antigen, or at least were set free from the organ cells and passed into the blood under this influence, more or less in analogy with genuine antibodies, from which, however, they were in many respects different.

But the North-American workers have made new and important researches on this point, which, tho not yet concluded, have in an eminent degree widened our views as to generalities in immunology.

The experiments of Jobling, Petersen and Eggstein, as well as of Bronfenbrenner, show that the placental tissue was not decomposed by the ferment, but on the contrary is augmented in weight as well as in nitrogenous substance, and if the placental tissue is digested in the cold with the serum and then separated from it by the centrifuge and both parts, placenta and salt solution, kept at 37 C., the serum is split and the placenta not. The only explanation of this is that the ferment exists in the serum but cannot develop its effect because of the presence of some body with antifermentative effect. This antiferment being adsorbed by the placenta, the ferment is free and able to digest the serum albumin substance.

[13] Schutzfermente des tierischen Organismus, 1912.

[14] Jobling, Petersen, and Eggstein: Jour. Exper. Med., 1915, 21, p. 239. Bronfenbrenner: Ibid., 1915, 21, p. 221.

The question whether the ferment exists always preformed in the serum is answered in the affirmative by Bronfenbrenner, but Jobling and his co-workers state that in human serum the ferment exists free only under certain circumstances, such as pregnancy and cancer. Normally it is contained in the leukocytes. In the blood of guinea-pigs and rabbits it exists, however, under normal conditions. But they agree that the ferment normally, tho free in the blood, is prevented from developing its effect by the existence of an antiferment, and such they consider the lipoid substances of the blood to be. They state moreover that every substance that is capable of adsorbing lipoids can also set free the ferment of the blood in which it exists.

Bronfenbrenner is of the opinion that the fixation of lipoids under fitting circumstances is perfectly analogous to the fixation of complement and takes place where a specific antigen unites with its specific antibody.

Under normal circumstances the blood ferment in vivo as well as in vitro is inactive, but we have in the anaphylactic shock a clinical picture that, as all workers agree, is due to the intoxication of the organism with toxic split products of albuminous substances resulting from the effect of some tryptic ferment.

The first to state this opinion was Friedberger,[15] who in a series of papers developed the doctrine of anaphylatoxin, but his opinion was that the antigen is split by the complement, after being sensitized by the amboceptor. But it soon became apparent that neither the immune body nor the antigen[16] was necessary for the splitting. Serum was split with kaolin (Keysser and Wassermann), agar (Besredka[17]), or other substances. Jobling and Petersen have made further experiments along this line. They state that the anaphylactic shock comes into existence through the union of antigen and antibody in vivo. The substance resulting is capable of adsorbing the lipoids in the serum, thus setting at liberty the tryptic ferment.

The relation of this ferment to the complement is not as yet clear. They cannot be identical, but it may be possible that the complement is a ferment of other nature and effect, a lipase (Jobling, Petersen, and Eggstein).

[15] Ztschr. f. Immunitätsf., 1912, 12, p. 241; 1913-14, 20, p. 405; 1913, 17, p. 506; 1912, 12, p. 241.

[16] Keysser and Wassermann: Ztschr. f. Hyg. u. Infektionskrankh., 1911, 68, p. 535.

[17] Compt. rend. Soc. de biol., 1911, 71, p. 413.

IX

After the preceding statements it appears probable that in the blood under normal as well as pathologic circumstances, in vitro as well as in vivo, there takes place a very complicated ensemble between several ferments and antiferments, all of them colloid substances whose degree of dispersity has great influence on their effect. And more, the equilibrium of these substances is a very labile one, so that a small change may give opportunity for the most far-reaching alterations.

So it may be possible that the colloidal changes which take place through the union of antigen and antibody may disturb the equilibrium of the whole system and inactive ferments of any nature may be activated or active ferments put out of action by adsorption to the new formed colloidal union.

If we can produce sufficient experimental support for this hypothesis, it may be found that the colloidal chemical relations are nothing more than an introduction to the real process, or figuratively speaking, the spring that starts the machine. As to the protective ferments of Abderhalden, the argument appears rather complete, but for the genuine processes of immunology we are still far away from this point.

I might venture to suggest the possibility that the pathogenesis of a great many of the infectious diseases may be explained from this point of view; namely, that the living bacteria or protozoa disturb the colloidal equilibrium of the liquids of the organism and so put normal ferments out of action or pathologic ferments into activity. Theoretically such a process appears very probable, but as yet we have no experimental support for it.

X

But still it may be possible that colloidal processes may be of some direct importance in immunologic phenomena. Bull[18] has discovered that agglutinations may on occasions take place in vivo. He studied rabbits with pneumococcemia. When he injected pneumococcal serum intravenously into these animals, he could immediately afterwards (for 30 seconds) observe an agglutination of pneumococci in the blood. But very soon the agglutinated bacteria were filtered from the blood by liver, spleen, and other organs. Here they were promptly taken up by the phagocytes, more promptly than pneumococci which had not been agglutinated. It may not be improbable that the phagocytosis itself is more related to colloidal phenomena than we think; the ameboid move-

[18] Jour. Exper. Med., 1915, 22, p. 457.

ments of the leukocytes as well as of the ameba are in reality due to local changes in their surface tensions, and it is probable that the action on bacteria and opsonin is such as to make them able to provoke such changes in the surface of the leukocyte when they touch it.

XI

If, finally, I try to summarize the relations between immunology and colloidal chemistry I shall first venture to assert that we surely shall be at fault if we neglect the colloidal chemical laws in immunologic researches. On the contrary we shall be more right if we concentrate our attention first on those laws and examine how much of the problem they will be able to explain.

But on the other hand it is quite clear that only a small part of immunology will be explained through alternations and adsorptions, the real process in most cases being quite another.

We therefore have always to consider the colloidal chemical part of our immunologic investigations only as preliminary, as a gate to the real process.

In vivo we do not encounter the colloidal chemical processes so pronouncedly as in vitro. The alternations and adsorptions observed play a small part only. What we observe is the effects of the changes in the liquids brought about by these processes. In conclusion I think I may be right in assuming that colloidal chemistry is an important factor in immunology tho but one factor of several. That as isolated method it does not explain anything at all, but as a help to disentangle the complicated processes concerned it may be of immense service.